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FRI0369

MIMICKING GLUCOCORTICOID-INDUCED OSTEOPOROSIS USING AN IN VITRO TRABECULAR HUMAN BONE MODEL

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Background: The bone matrix consists of inorganic and organic components and a variety of specialized cells such as osteoblasts, osteocytes and osteoclasts. The bone-forming osteoblasts are responsible for the production of organic matrix components; they differentiate later into osteocytes which is accompanied by matrix mineralization. Osteoclasts are multinuclear giant cells, which resorb bone. Healthy bone homeostasis is characterized by a balanced, dynamic and continuous remodeling process. Glucocorticoids (GCs) are commonly used to successfully treat patients with inflammatory rheumatic and other autoimmune diseases. However, long-term treatment with GC can potentially lead to several adverse effects such as the inhibition of osteoblast proliferation and the increase of osteoclastic activity resulting in osteoporosis.

Objectives: Hence, the aim of our project is to i) develop an *in vitro* trabecular human bone model, ii) integrate this bone model into a perfusion system to accelerate mineralization and provide biomechanical stimuli and iii) applying prednisolone to induce osteoporosis. Here we present our initial results describing the successful differentiation of osteoblasts and osteoclasts in a 3D environment, and the accomplished integration of the bone model into a perfusion system.

Methods: In a first step, different cultivation conditions were tested to allow optimal osteogenic or osteoclastic differentiation. To this end, a) human bone marrow derived mesenchymal stromal cells (hMSCs) were treated with osteogenic medium, and b) monocytes (isolated from buffy coats) were differentiated into osteoclasts using following protocol: incubation for 3 days with 25 ng/ml M-CSF followed by an 18-day incubation with M-CSF and 50 ng/ml RANKL. Calcification of hMSCs was evaluated via Alizarin Red S staining. Osteoclasts were identified using immunofluorescence staining observing multinucleated (DAPI) giant (β -Actin) cells with TRAP and Cathepsin K activity. Additional gene expression analyses are currently conducted using qRT-PCR and looking for osteoclast-specific genes. In parallel to the monolayer cultures, cells were transferred on β -tricalcium phosphate (β TCP) – a suitable bony-like scaffold. Furthermore, first experiments in a dynamic bioreactor platform (OSPIN GmH) were conducted to evaluate the influence of shear stress on the cells and model systems.

Results: We have been able to populate the β TCP scaffold with monocytes, which were differentiated into osteoclasts (morphological changes) without any effect on cellular viability as measured by Live/Dead staining. The morphological changes of those osteoclasts such as formation of filopodia could be demonstrated by scanning electron microscopy. In addition, the cultivation of β TCP populated with hMSCs in a perfusion system showed the upregulation of osteogenic markers (RUNX2, OSX) on mRNA-level.

Conclusion: These first results of our approach to develop an *in vitro* 3D model for glucocorticoid-induced osteoporosis are promising. Our next step will be the co-cultivation of osteoblasts and osteoclasts under dynamic and optimized cultivation conditions. By combining several cell types, a suitable scaffold and biomechanical stimuli (perfusion), we aim to provide a valid testing platform to study underlying disease mechanisms and for drug development.

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FRI0370

DUAL OXIDASE MATURATION FACTOR 1 POSITIVELY REGULATES RANKL-INDUCED OSTEOCLASTOGENESIS VIA ACTIVATING REACTIVE OXYGEN SPECIES PRODUCTION AND TRAF6-MEDIATED SIGNALING

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Background: Reactive oxygen species (ROS) are one of the significant factors of chemical or physical cell signaling in a wide variety of cell types including skeletal cells. Receptor activator of NF- β B ligand (RANKL) induces generation of intracellular ROS, which act as second messengers in RANKL-mediated osteoclastogenesis. Dual oxidase maturation factor 1 (Duoxa1) was first identified as a *Drosophila* Numb-interacting protein (NIP), and has been associated with the maturation of ROS generating enzymes including dual oxidases (Duox1 and Duox2). In the progression of osteoclast differentiation using mouse bone marrow-derived macrophages (BMMs), we identified that only Duoxa1 level showed an effective change upon RANKL stimulation, but not Duox1, Duox2, and Duoxa2.

Objectives: we hypothesized that Duoxa1 could independently act as a second messenger for RANKL stimulation and regulate ROS production during osteoclast differentiation.

Methods: Using siRNA or retrovirus transduction and knockdown of Duoxa1 via siRNA

Results: Duoxa1 level gradually increased during RANKL-induced osteoclast differentiation. We found that Duoxa1 regulated RANKL-stimulated osteoclast formation and bone resorption positively. Knockdown of Duoxa1 via siRNA decreased the RANKL-induced ROS production. During Duoxa1-related control of osteoclastogenesis, activation of tumor necrosis factor receptor (TNFR)-associated factor 6 (TRAF6)-mediated early signaling molecules including MAPKs, Akt, β B, Btk, and PLC 2 was affected, which sequentially modified the mRNA or protein expression levels of key transcription factors in osteoclastogenesis, such as c-Fos and NFATc1, as well as mRNA expression of osteoclast-specific markers including OSCAR, ATP6v0d2, and CtSK.

Conclusion: Overall, our data indicate that Duoxa1 plays a crucial role in osteoclastogenesis via regulating RANKL-induced intracellular ROS production and activating TRAF6-mediated signaling.

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FRI0371

BETULINIC ACID INHIBITS RANKL-INDUCED OSTEOCLASTOGENESIS VIA ATTENUATING AKT, NF-KB, AND PLCG2-CA2+ SIGNALING AND PREVENTS INFLAMMATORY BONE LOSS

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Background: Betulinic acid (BA), a natural plant-derived pentacyclic triterpenoid compound, is known to possess numerous pharmacological and biochemical properties including anti-inflammatory, anti-cancer, and anti-adipogenic activity.

Objectives: we investigated that BA could suppress RANKL-induced osteoclastogenesis and bone resorption.

Results: BA significantly suppressed osteoclastogenesis by decreasing the phosphorylation of Akt and I κ B, as well as PLC γ 2-Ca²⁺ signaling, in pathways involved in early osteoclastogenesis as well as through the subsequent suppression of c-Fos and NFATc1. The inhibition of these pathways by BA was once more confirmed by retrovirus infection of constitutively active (CA)-Akt and CA-I κ B β retrovirus and measurement of Ca²⁺ influx. BA also significantly inhibited the expression of osteoclastogenesis-specific marker genes. Moreover, we found that BA administration restored the bone loss induced through acute lipopolysaccharide injection in mice by a micro-CT and histological analysis.

Conclusion: Our findings suggest that BA is a potential therapeutic candidate for bone diseases involving osteoclasts.

Disclosure of Interests: None declared

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FRI0372 INCREASED EXPRESSION OF NOTCH RECEPTORS ON OSTEOCLAST PROGENITORS INDUCED BY RHEUMATOID ARTHRITIS

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Background: Systemic and periarticular bone loss in rheumatoid arthritis (RA) is mediated by osteoclasts, multinucleated cells originating from the myeloid lineage. Recently, Notch signaling pathway has emerged as a potential regulator of osteoclast progenitor (OCP) differentiation and activation.

Objectives: The exact role of Notch signaling in the context of arthritis is still unknown; however, its inhibition has beneficial effects in animal arthritis models. We aimed to determine the expression of Notch receptors and ligands on specific OCP subpopulations and define changes that occur in murine collagen-induced arthritis (CIA) and RA patients.

Methods: Peripheral blood, synovial tissue and subchondral bone marrow were collected from RA patients, and periarticular bone marrow (PBM) and spleen (SPL) were harvested from male C57/Bl6 mice immunized with chicken type II collagen. Notch 1 to 4 receptor expression on OCPs was analyzed by flow cytometry. Gene expression of Notch receptors/ligands was determined by qPCR from tissues and sorted OCPs. Sorted OCPs were cultured, with addition of MCSF and RANKL, in control, IgG, Jagged (Jag) 1 or Delta (DLL) 1 coated wells. Immunohistochemistry (IHC) for Notch 1 and 2 was performed on sections of murine hind paws. Research was approved by Ethics committee.

Results: We previously identified peripheral and periarticular subpopulations of murine and human OCPs, as CD45⁺CD3⁺B220⁺NK1.1⁺CD11b^{lo/hi}CD115⁺CCR2⁺ and CD45⁺CD3⁺CD19⁺CD56⁺CD11b⁺CD14⁺CCR2⁺ respectively, specifically associated with arthritis. Flow cytometry revealed that majority of murine splenic and periarticular OCPs express Notch 2, whereas Notch 1 and 4 were expressed on approximately 10% of cells. In CIA, this highly osteoclastogenic population is expanded as is the expression of Notch 4 in PBM and Notch 3 in SPL. Majority of human peripheral-blood OCPs express Notch 2 and 4, with a specific increase in the expression of Notch 1 and 3 in RA. In contrast, RA synovial-derived OCPs

mostly express Notch 1 to 3, whereas subchondral OCPs mostly express Notch 1 and 4. Notch ligands were analyzed at mRNA level and revealed expression of Jag1, Jag2 and DLL4 in murine sorted OCPs and Jag1 and DLL1 in human sorted OCPs. Expression of Notch 1 and 2 was confirmed by IHC on arthritic murine hind paws, with Notch 2 expressed by bone marrow, synovial tissue and chondrocytes and Notch 1 expressed by chondrocytes and synovial tissue. Increased expression of Notch 1, Notch 2 and Jag1 was also confirmed in murine arthritic periarticular tissue by qPCR. During osteoclastogenic culture, murine and human OCPs exhibit a similar gene expression pattern with higher initial expression of Notch 1 and 2, and increase in the expression of Notch 3 and 4 with differentiation. Osteoclasts were also differentiated under Notch-ligand stimulation. Coating with DLL1 results in a greater number of cells expressing osteoclast-specific TRAP, whereas Jag1 seemed to inhibit osteoclastogenesis.

Conclusion: Our results indicate that murine and human OCPs express a distinct tissue-specific pattern of Notch receptors. Notch signaling in OCPs is increased in arthritis and may contribute to the osteoclastogenic potential and increased bone resorption. Our next aim would be to determine the effect of Notch inhibition on OCP activity and arthritis severity.

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FRI0373 ASSOCIATIONS OF VASCULAR PATHOPHYSIOLOGY AND BONE METABOLISM IN ANTI-TNF- TREATED RHEUMATOID ARTHRITIS AND ANKYLOSING SPONDYLITIS PATIENTS

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Background: Cardiovascular (CV) disease and osteoporosis (OP) have become increasing challenges in the ageing population, even more in patients with inflammatory rheumatic diseases, such as rheumatoid arthritis (RA) and spondyloarthropathies. Both RA and ankylosing spondylitis (AS) have been associated with generalized and localized bone loss, accelerated atherosclerosis, increased CV morbidity and mortality.

Objectives: Bone and vascular biomarkers and parameters along with the effect of one-year anti-TNF therapy on these markers were assessed in order to determine correlations between vascular pathophysiology and bone metabolism in RA and AS.

Methods: Fifty-three patients including 36 RA patients treated with etanercept (ETN) or certolizumab pegol (CZP) and 17 AS patients treated with ETN were included in a 12-month follow-up study. Bone and vascular markers were assessed by ELISA. Bone density was assessed by DXA and quantitative CT (QCT). Flow-mediated vasodilation (FMD), common carotid intima-media thickness (ccIMT) and pulse-wave velocity (PWV) were assessed by ultrasound. The effects of vascular markers on bone and bone effects on vasculature undergone statistical analysis.

Results: Serum levels of vascular endothelial growth factor (VEGF), PDGF-BB, angiopoietin 2 (Ang2) and cathepsin K (CathK) decreased, procollagen type 1 N-propeptide (P1NP) and sclerostin (SOST) levels increased, soluble receptor activator nuclear kappa B ligand (sRANKL) and osteoprotegerin (OPG) levels showed no differences. When bone and vascular markers were correlated with each other, at baseline, OPG correlated with Ang2 and adiponectin. SOST correlated positively with ccIMT. DXA L2-4 BMD, DXA L1 BMD and DXA femoral neck (FN) BMD correlated with FMD and CRP. QCT trabecular BMD correlated with ccIMT and PON1. According to the univariate analysis, FMD correlated with OPG, ccIMT correlated with SOST and QCT trabecular BMD. Ang1, Ang2 and PDGF-BB showed correlation with Dickkopf-1 (DKK1). Ang2 also correlated with OPG. As suggested by the multivariate analysis, OPG determined FMD; DKK1 was an independent predictor of Ang1, Ang2 and PDGF-BB. OPG was a predictor of Ang2.

Conclusion: In our study of anti-TNF treated RA and AS patients, vascular and bone parameters showed numerous correlations. The therapy was clinically effective, it halted further bone loss over 1 year and reduced the production of angiogenic markers.

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